

CLAIMS

1/ Composition of polypeptides, characterized in that it contains at least one protein or part of a protein selected from the amino acid sequences identified in the list of the sequences as SED ID NO 1 (VanH), SEQ ID NO 2 (VanA), SEQ ID NO 3 (VanX) or SEQ ID NO 19 (VanC) or any protein or part of a protein recognized by the antibodies directed against VanH, VanA, VanX or VanC or any protein or part of a protein encoded by a sequence hybridizing with one of the nucleotide sequences identified in the list of the sequences as SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10 or SEQ ID NO 21 or with one of the following sequences V1 or V2 under stringent or only slightly stringent conditions:

V1 : GGX GAA GAT GGX TCX TTX CAA GGX

G C AG C G
A

V2 : AAT ACX ATX CCX GGX TTT AC

C T C
C

- 2/ Composition of polypeptides according to Claim 1, characterized in that it contains at least 3 proteins or any part of one or more of these proteins necessary to confer on Gram-positive bacteria resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin or to promote this resistance, in particular in strains of the family of the Gram-positive cocci, these proteins or parts of proteins being
- either recognized by antibodies directed against one of the sequences identified in the list of the sequences as SEQ ID NO 1 (VanH), SEQ ID NO 2 (VanA), SEQ ID NO 3 (VanX),
 - or encoded in genes containing a sequence identified as SEQ ID NO 8, SEQ ID NO 9 or SEQ ID NO 10 or hybridizing with one of these sequences or its complementary sequence or with the sequences V1 or V2 under stringent or only slightly stringent conditions.
- 3/ Composition of polypeptides according to Claim 1 or 2, characterized in that it corresponds to the combination of the proteins designated

as SEQ ID NO 1 (VanH), SEQ ID NO 2 (VanA), SEQ ID NO 3 (VanX).

4/ Composition of polypeptides according to Claim 2 or Claim 3, characterized in that the VanC protein corresponding to the sequence SEQ ID NO 19 replaces the VanA protein corresponding to the sequence SEQ ID NO 2.

5/ Composition of polypeptides according to any one of the Claims 1 to 4, characterized in that the amino acid sequences necessary for the expression of resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin are under the control of regulatory elements, in particular proteins corresponding to the sequences designated as SEQ ID NO 4 (VanR) or SEQ ID NO 5 (VanS) in the list of the sequences.

6/ Composition according to any one of the Claims 1 to 5, characterized in that it is encoded in one of the sequences SEQ ID NO 6, SEQ ID NO 11, SEQ ID NO 22 identified in the list of the sequences.

~~7/ Purified protein characterized in that it corresponds to the sequence SEQ ID NO 2 (VanA) or to the sequence SEQ ID NO 19 (VanC), contained in the composition according to ^{claim 1} ~~any one of the Claims 1 to 3.~~~~

8/ Protein characterized in that it corresponds to one of the sequences identified as SEQ ID NO 1 (VanH), SEQ ID NO 3 (VanX), SEQ ID NO 4 (VanR), SEQ ID NO 5 (VanS).

9/ Nucleotide sequence characterized in that it codes for an amino acid sequence according to any one of the Claims 1 to 8, or in that it is a complementary DNA sequence or a corresponding RNA sequence.

10/ Nucleotide sequence of about 7.3 kb, corresponding to the HindIII-EcoRI restriction fragment as obtained from the plasmid pIP816 comprising this HindIII-EcoRI fragment or any part of this fragment, in particular the 3.4 kb EcoRI-XbaI fragment, the EcoRV-SacII fragment of about 1.7 kb and the 3.3 kb HindIII-EcoRI fragment.

11/ Nucleotide sequence according to Claim 10, characterized in that it contains the following restriction sites as obtained from the plasmid pIP816 in the order:

HindIII, BglII, BglII, EcoRI, BamHI, XbaI, EcoRI

12/ Nucleotide sequence according to any one of the Claims 8 to 10,

characterized in that it corresponds to one of the sequences identified as SEQ ID NO 6, SEQ ID NO 7 or SEQ ID NO 22, or in that it includes one of these sequences or any part of one of these sequences or also any sequence or part of a sequence of complementary DNA, or any RNA sequence corresponding to one of these DNAs, capable of

- either constituting a hybridization probe or primer for the detection of resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin in particular in strains of the family of the Gram-positive cocci,

- or of coding for a sequence necessary for the expression or regulation of resistance to antibiotics of the glycopeptide family, in particular to vancomycin and/or teicoplanin in particular in strains of the family of the Gram-positive cocci.

13/ Nucleotide sequence according to Claim 12, characterized in that it includes or in that it corresponds to one of the following sequences:

V1 : GGX GAA GAT GGX TCX TTX CAA GGX

G C AG C G
A

V2 : AAT ACX ATX CCX GGX TTT AC

C T C
C

14/ Nucleotide sequence according to any one of the Claims 10 to 12, characterized in that it is one of the sequences SEQ ID NO 8 (vanA), SEQ ID NO 9 (vanH), SEQ ID NO 10 (vanX), SEQ ID NO 21 (vanC), SEQ ID NO 12 (transposase), SEQ ID NO 13 (resolvase), SEQ ID NO 14 (vanY), SEQ ID NO 15 (vanZ), SEQ ID NO 23 (vanR), SEQ ID NO 24 (vanS) or any variant of one of these sequences provided that it codes for a protein having immunological and/or functional properties similar to those of the proteins encoded in the sequences SEQ ID NO 8 (vanA), SEQ ID NO 9 (vanH), SEQ ID NO 10 (vanX), SEQ ID NO 21 (vanC), SEQ ID NO 12 (transposase), SEQ ID NO 13 (resolvase), SEQ ID NO 14 (vanY), SEQ ID NO 15 (vanZ), SEQ ID NO 23 (vanR), SEQ ID NO 24 (vanS), or provided that they make possible the detection of strains resistant to antibiotics of the glycopeptide family.

15 / Nucleotide sequence according to any one of the Claims 9 to 12, characterized in that it corresponds to the sequence SEQ ID NO 6 or to the sequence SEQ ID NO 22 or in that it includes this sequence.

16 / Recombinant sequence, characterized in that it includes a sequence of nucleotides according to any one of the Claims 9 to 14 under the control of regulatory elements capable of contributing to the expression of resistance to antibiotics of the glycopeptide family, in particular to vancomycin or teicoplanin in a specific host.

17 / Recombinant vector, characterized in that it includes a nucleotide sequence according to any one of the Claims 9 to 16, at a site inessential for its replication under the control of regulatory elements capable of contributing to the expression of resistance to antibiotics of the glycopeptide family, in particular to vancomycin or teicoplanin, in a specific host.

18/ Recombinant vector according to Claim 17, characterized in that it is the plasmid pAT214.

19/ Recombinant cell host, characterized in that it includes a nucleotide sequence according to any one of the Claims 9 to 16 or a vector according to Claim 17 or Claim 18 under conditions leading to the expression of resistance to antibiotics of the glycopeptide family, in particular to vancomycin or teicoplanin, this host being for example selected from the bacteria, in particular from the Gram-positive cocci.

20/ Nucleotide probe, characterized in that it is a DNA or a RNA and in that it is capable of hybridizing with a sequence according to any one of the Claims 9 to 15, this probe being if necessary labelled, for example it is one of the nucleotides:

V1 : GGX GAA GAT GGX TCX TTX CAA GGX

G C AG C G

A

V2 : AAT ACX ATX CCX GGX TTT AC

C T C

C

21/ Nucleotide probe according to Claim 19, characterized in that it is specific for the sequences in Gram-positive bacteria encoding

a protein for resistance to glycopeptides, in particular to vancomycin and/or teicoplanin and is universal among these sequences.

22/ Nucleotide probe according to Claim 20, characterized in that it is specific for a nucleotide sequence coding for a protein necessary for the expression of high-level resistance to antibiotics of the glycopeptide family, in particular to vancomycin and teicoplanin in Gram-positive bacteria.

23/ Nucleotide probe according to Claim 20, characterized in that it is specific for a nucleotide sequence coding for a protein necessary for the expression of low-level resistance to antibiotics of the glycopeptide family, in particular to vancomycin in Gram-positive bacteria.

24/ Nucleotide probe according to any one of the Claims 20 to 23, characterized in that it hybridizes with a non-chromosomal nucleotide sequence of a strain resistant to glycopeptides, in particular to vancomycin and/or teicoplanin in particular that it hybridizes with a non-chromosomal nucleotide sequence of a strain of Gram-positive cocci, for example a strain of enterococci and preferably E. faecium 4147.

25/ Polyclonal or monoclonal antibodies, characterized in that they recognize the composition according to any one of the Claims 1 to 6 or an amino acid sequence according to any one of the Claims 7 or 8.

26/ Kit for the in vitro diagnosis in a biological sample of the presence of strains resistant to the glycopeptides, in particular to vancomycin and/or teicoplanin these strains belonging in particular to the Gram-positive cocci, in particular in that they are strains of enterococci, for example E. faecium, characterized in that it contains:

- antibodies according to Claim 25, labelled if necessary,
- a reagent for the detection of an immunological reaction of the antigen-antibody type,
- where appropriate, reagents to effect the lysis of the cells in the sample to be tested.

27/ Kit for the in vitro diagnosis of the presence of strains resistant to the glycopeptides, in particular resistant to vancomycin and/or

to teicoplanin these strains belonging in particular to the Gram-positive cocci, in particular in that they are strains of enterococci, for example E. faecium, characterized in that it contains:

- a nucleotide probe according to any one of the Claims 20 to 24, and if necessary,
- oligonucleoside triphosphates dATP, dCTP, dTTP, dGTP,
- an agent for the polymerization of DNA.

28/ Procedure for the in vitro detection of the presence of strains resistant to the glycopeptides, in particular to vancomycin and/or teicoplanin these strains belonging in particular to the family of the Gram-positive cocci, in particular in that they are strains of enterococci, for example E. faecium or E. gallinarum. characterized in that it comprises:

- a) the placing of a biological sample likely to contain the resistant strains in contact with a primer constituted by a nucleotide sequence according to any one of the Claims 20 to 24, which is capable of hybridizing with the nucleotide sequence under investigation, necessary for the expression of resistance, this sequence being used as matrix in the presence of the 4 different nucleoside triphosphates and a polymerization agent under hybridization conditions such that for each nucleotide sequence which has hybridized with a primer, an elongation product of each primer is synthesized which is complementary to the matrix,
- b) the separation of the matrix from the elongation product obtained, this latter being then also able to serve as matrix,
- c) the repetition of step a) so as to produce a detectable amount of the nucleotide sequences under investigation,
- d) the detection of the amplification product of the nucleotide sequences.

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